DATA EVALUATION RECORD
§72-3(B) -- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

Data Requirement:	PMRA Data Code	9.4.4
	EPA DP Barcode	DP349851
	OECD Data Point	IIA 8.11.1
	EPA MRID	47127902
	EPA Guideline	OPPTS 850.1025

1. **CHEMICAL:** Saflufenacil — PC Code No.: 118203

2. **TEST MATERIAL:** BAS 800 H — Purity: 93.8%

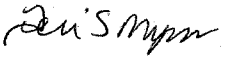
3. **CITATION**

Authors:	Palmer, S.J., T.Z. Kendall, H.O. Krueger and C. Holmes.
Title:	BAS 800 H: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
Study Completion Date:	November 5, 2007
Laboratory:	Wildlife International, Ltd., Easton, MD
Sponsor:	BASF Corporation, Research Triangle Park, NC
Laboratory Report ID:	147A-214
MRID No.:	471279-02
DP Barcode:	DP349851

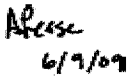
4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 03/24/08

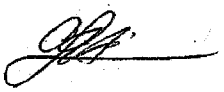
APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 04/04/08

5. **APPROVED BY:** Primary Reviewer: Anita Pease, Senior Biologist, U.S. EPA

Signature:  **Date:** 06/09/09

Secondary Reviewer: Ann Lee, HC-PMRA-EAD

Signature:  **Date:** 06/09/09

Secondary Reviewer: Farzard Jahromi, DEWHA-APVMA

Signature:  **Date:** 06/09/09

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shell deposition in oysters. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. EXECUTIVE SUMMARY: In a 96-h shell deposition study, Eastern oyster (*Crassostrea virginica*), were exposed nominal saflufenacil (BAS 800 H) concentrations of 0 (negative and dimethyl formamide solvent control), 0.78, 1.3, 2.2, 3.6, and 6.0 mg a.i./L. TWA concentrations were <0.3 (<LOQ for negative and solvent controls), 0.806, 1.36, 2.32, 3.65, and 6.08 mg a.i./L, respectively. The reviewer's results are similar to those reported by the study author; however, the reviewer's results are based on time-weighted average (TWA) concentrations, whereas the study author's were based on mean-measured concentrations.

At 96 h, no moralitites occurred and mean shell deposition was greater in all treatment levels relative to the negative control. Therefore, the observed 96-h EC_{50}, based on the TWA concentrations, was >6.08 mg a.i./L and the NOAEC value was 6.08 mg a.i./L. Based on the results of this study, BAS 800 H is categorized as practically non-toxic to the Eastern oyster at the limit of its solubility on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an acute shell deposition study with Eastern oyster.

7. STUDY PARAMETERS

Age or Size of Test Organism:	42.1 (34.0-48.7) mm (N=20)
Definitive Test Duration:	96 hours
Study Method:	Flow-Through

Type of Concentrations: Time-Weighted Average (TWA)

8. CONCLUSIONS:

Based on the results of this study, Saflufenacil is categorized as practically nontoxic to mollusks on an acute toxicity basis up to the level tested in this study.

Results Synopsis
EC_{50}: >6.08 mg a.i./L (TWA) 95% C.I.: N/A
NOAEC: 6.08 mg a.i./L (TWA)
Probit Slope: N/A

9. ADEQUACY OF THE STUDY

A. Classification: ACCEPTABLE to U.S. EPA; FULLY RELIABLE to PMRA and APVMA

B. Rationale: For mean shell deposition, there was a significant difference between the negative and solvent control groups. According to the EPA memo titled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, a significant difference between the negative and solvent control groups may result in an INVALID study classification. However, given that no mortalities occurred and the mean shell deposition was greater at all treatment levels relative to the negative control, the differences between the negative and solvent control do not affect the acceptability of this study. In addition, comparison of treatment groups to the solvent control shows that less than 50% inhibition of growth was observed in all treatment levels.

C. Repairability: NA

10. BACKGROUND

11. GUIDELINE DEVIATIONS

1. It was not reported if all oysters were from the same year class.
2. The TOC of the dilution water was not reported.
3. A significant difference existed between the negative and solvent controls for mean shell deposition. Shell deposition was promoted by 22% in the solvent control, as compared to the negative control.

The significant difference between the solvent and negative controls for mean shell deposition does not affect the acceptability of this study because no mortalities occurred and the mean shell deposition was greater at all treatment levels relative to the negative control.

12. **SUBMISSION PURPOSE:** This study was submitted to provide data on the effects of the test material on the shell deposition of the eastern oyster (*Crassostrea virginica*) following acute laboratory exposure for the purpose of new chemical registration.

13. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	42.1±3.6 mm
Supplier	Circle C Oyster Ranch, Ridge, Maryland
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not Reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	10 days
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
Were there signs of disease or injury?	During acclimation, no signs of disease or stress were noted.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	The amount of peripheral shell growth removed was not specified; however, adequate shell growth was removed so that all oysters were within the 30-50 mm size criterion. The length was determined by measuring the longest distance from the umbo to the distal valve edge.
Feeding during the acclimation Must be fed to avoid stress.	Oysters received a suspension of microalgae (Reed Mariculture, Campbell, California) at a nominal rate of $2.9x10^9$ cells/oyster/day. During definitive testing, the rate was increased to $5.8x10^9$ cells/oyster/day.
Pretest Mortality <3% mortality 48 hours prior to testing	No pre-test mortality was reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural seawater collected at Indian River Inlet, Delaware that was filtered and diluted with well water.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34‰ (parts per thousand) salinity, weekly	Adjusted to 20 ‰

Guideline Criteria	Reported Information
range < 6 ‰	
Water Temperature 15°-30°C, consistent in all test vessels	19.5-21.5°C
pH	7.8-8.0
Dissolved Oxygen ≥60% throughout	5.9-7.0 mg/L; 4.8 mg/L represents 60% saturation at 20°C in saltwater with a salinity of 20‰.
Total Organic Carbon	Not Reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass aquaria, volume of 54 L filled with approximately 27 L of test water. Water depth was approximately 18 cm and test chambers were maintained in a temperature-controlled environmental chamber.
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter. A syringe pump delivered the appropriate stock solution into mixing chambers containing dilution water, where the appropriate concentrations were obtained and delivered to the test vessels. The general operation of the diluter was checked visually at least twice each day during the test and at least once on the last day of the test.
Flow rate Consistent flow rate	Approximately 19 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes. Oysters were placed such that flat valves were facing up and umbos away from the water.
Photoperiod 16 hours light, 8 hours dark	16L:8D with a 30 minute transition period of low-light intensity. Light intensity at test

Guideline Criteria	Reported Information
	initiation was 195 lux at the surface of the water from one representative test chamber.
Solvents Not to exceed 0.5 ml/L	Solvent: dimethyl formamide (DMF) Maximum conc.: 0.1 ml/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	A preliminary non-GLP range-finding test was conducted at nominal concentrations ranging from 0.24 to 30 mg a.i./L. There were no effects on shell deposition up to 9.0 mg a.i./L, and only 27% inhibition at 30 mg a.i./L. However, a white precipitate was observed in the diluter mixing chambers at 9.0 and 30 mg a.i./L and in the test chambers at 30 mg a.i./L. Therefore, the highest nominal concentration for the definitive test was selected to test up to the apparent limit of solubility in the test system.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	0 (negative and solvent controls), 0.78, 1.3, 2.2, 3.6 and 6.0 mg a.i./L TWA: <0.300 (<LOQ; controls), 0.806, 1.36, 2.32, 3.65 and 6.08 mg a.i./L
Number of Test Organisms Minimum 20 individual per test level and in each control	20 per control and treatment level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24	Yes

Guideline Criteria	Reported Information
hours?	
Water Parameter Measurements	
1. Temperature Measured hourly in at least one chamber	1. Temperature was measured in all test vessels at 0 and 96 hours, and continuously in the negative control.
2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	2. DO was measured in every test vessel at 0, 24, 48, 72 and 96 hours. pH was measured in every test vessel at 0, 48 and 96 hours.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

14. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with the Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau 1, October 1999), with the following exception: periodic analyses of saltwater for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.

Guideline Criteria	Reported Information
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% in both controls
Control Shell Deposition Must be at least 2 mm.	4.33 and 5.28 mm in the negative and solvent controls, respectively.
Recovery of Chemical	101.3-105.3% of nominal, based on the reviewer-calculated TWA concentrations (see Appendix II).
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (mg a.i./L)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction[1]	
Nominal	TWA				Relative to Negative Control	Relative to Solvent Control
Control	<0.300	20	0	4.33	N/A	18.0
Solvent Control	<0.300	20	0	5.28	-21.9	N/A
0.78	0.806	20	0	5.36	-23.8	-1.51
1.3	1.36	20	0	4.50	-3.9	14.8
2.2	2.32	20	0	5.39	-24.5	-2.08
3.6	3.65	20	0	4.40	-1.6	16.7
6.0	6.08	20	0	5.02	-15.9	4.92

[1] Inhibitions were reviewer-calculated relative to the negative and solvent controls. Negative

inhibitions indicate promoted growth.

B. Statistical Results

Method: Statistical analyses were conducted using the TOXSTAT® computer program. Negative and solvent control shell deposition data were compared using an appropriate t-test. There was a significant difference between the control groups ($p \leq 0.05$). Therefore, growth in the treatment groups was compared to the solvent control data. The shell deposition data were evaluated for normality and homogeneity of variance using the Chi-square test and Bartlett's test, respectively. Since the data passed the assumptions of normality and homogeneity, the data in the treatment groups were compared to the solvent control data using analysis of variance (ANOVA) and Dunnett's test to identify any significant differences. The NOAEC was determined from the statistical analysis of the data and an assessment of the concentration-response pattern. There was less than 50% inhibition of growth in any treatment group in comparison to the solvent control. Therefore, the EC_{50} was estimated to be greater than the highest concentration tested.

96-hr EC_{50}: >6.0 mg a.i./L 95% C.I.: N/A
NOAEC: 6.0 mg a.i./L
Probit Slope: N/A

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	Visual determination. Shell growth was promoted at all levels relative to the negative control
EC_{50} (95% C.I.)	>6.08 mg a.i./L (TWA)
Probit Slope	N/A
Statistical Method for NOAEC	Visual determination. Shell growth was promoted at all levels relative to the negative control.
NOAEC	6.08 mg a.i./L (TWA)

16. REVIEWER'S COMMENTS:

The reviewer compared the replicate shell deposition data from the negative and solvent controls using a t-Test assuming equal variance via Microsoft Excel (refer to Appendix I) and detected a significant difference ($p \leq 0.05$). Shell deposition in the solvent control was 21.9% greater than the mean shell deposition in the negative control. No mortalities occurred and mean shell deposition was greater at all treatment levels relative to the negative control, therefore, the reviewer visually determined all toxicity values. The reviewer's results were determined based on the time-weighted average (TWA) concentrations (refer to associated Excel worksheet in Appendix II), while those of the study authors were determined based on the nominal concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer determined the time-weighted average concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours)

For mean shell deposition, there was a significant difference between the negative and solvent control groups, which according to the EPA memo titled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, could result in the INVALID classification of this study. However, given that no mortalities occurred and the mean shell deposition was greater at all treatment levels relative to the negative control, the differences between the negative and solvent control do not affect the acceptability of this study. As specified in EPA's guidance memo on statistical analysis for guideline aquatic toxicology studies, the dilution water data (i.e., negative control) should be used as the control for comparison to treatment groups. However, comparison of treatment groups to the solvent control shows that less than 50% inhibition of growth was observed in all treatment levels; therefore, the EC_{50} value, relative to the solvent control, is also estimated to be greater than the highest concentration tested.

Analysis of pesticides, organics, and metals in the dilution water was performed, and raw data were

provided as part of the study. The results from the periodic screening analysis of the dilution water indicated the presence of the following constituents: barium (0.0074 mg/L), bromide (38.9 mg/L), calcium (228 mg/L), chloride (12,100 mg/L), magnesium (779 mg/L), potassium (269 mg/L), sodium (5,940 mg/L), and sulfate (1,500 mg/L).

All solutions appeared clear and colorless in the diluting mixing chambers and in the test chambers at test initiation. At test termination, solutions in the diluter mixing and test chambers appeared slightly cloudy green, due to algal feed, and green waste was observed on the bottom of the test chamber tanks.

The in-life portion of the definitive toxicity test was conducted from May 15 to May 19, 2006.

APPENDIX I. OUTPUT OF REVIEWER'S T-TEST VERIFICATION:

t-Test: Two-Sample Assuming Equal Variances

Control Shell Deposition (mm)	*Negative Control*	*Solvent Control*
Mean	4.33	5.275
Variance	1.123263158	0.628289474
Observations	20	20
Pooled Variance	0.875776316	
Hypothesized Mean Difference	0	
df	38	
t Stat	-3.193266835	
P(T<=t) one-tail	0.001412444	
t Critical one-tail	1.685954461	
P(T<=t) two-tail	**0.002824887**	
t Critical two-tail	2.024394147	

APPENDIX II. COPY OF REVIEWER'S TWA CALCULATIONS:

Time-Weighted Average (TWA) Concentrations

Nominal (mg ai/L)	0 Hrs	% of Nom.	48 Hrs	% of Nom.	96 Hrs	% of Nom.	TWA (mg ai/L)	% of Nom.
Negative Control	<0.300	N/A	<0.300	N/A	<0.300	N/A	<0.300	N/A
Solvent Control	<0.300	N/A	<0.300	N/A	<0.300	N/A	<0.300	N/A
0.78	0.766	98.2	0.827	106.0	0.804	103.1	0.806	103.3
1.3	1.36	104.6	1.37	105.4	1.34	103.1	1.36	104.6
2.2	2.24	101.8	2.36	107.3	2.31	105.0	2.32	105.3
3.6	3.57	99.2	3.71	103.1	3.60	100.0	3.65	101.3
6.0	5.82	97.0	6.20	103.3	6.08	101.3	6.08	101.3